PATENT APPLICATION

In the name of: DIGIBIO SA

For:	AN AMPLIFICATION PROCESS OF THE FORMATION OF LIGAND-RECEPTOR COMPLEXES AND ITS USES

Invention:

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AN AMPLIFICATION PROCESS OF THE FORMATION OF LIGAND-RECEPTOR COMPLEXES AND ITS USES

The present invention relates to an amplification process of the formation of complexes between the two elements of a ligand-receptor pair, to a process and to a device for detecting the presence, in a sample (hereinafter "analytical sample"), of a substance corresponding to one of the two elements of a ligand-receptor pair, implementing this amplification process, to the applications of this detection process, and to a process for detecting the presence, in an electromagnetic signal, of the electromagnetic signal characteristic of the biological activity of a substance corresponding to one of the two elements of a ligand-receptor pair, also implementing said amplification process.

To detect the presence of a substance in an analytical sample, very many methods have been suggested based on the capacity of this substance to bind itself specifically to one substance and to react with it.

In particular, the affinity properties presented by antibodies in respect of antigens are at the basis of a great number of immunological detection methods which in common use the formation of antigen-antibody complexes - the substance sought being able to be either the antigen, or the antibody - and detect, indeed quantify, the complexes so formed.

As examples of immunological detection methods which are very frequently used, may be mentioned immunoprecipitation, agglutination reactions, equilibrium dialysis, fluorescence suppression, fluorescence polarisation, immunoelectrophoresis, counter immunoelectrophoresis or electrosyneresis, radioimmunoassay

(FIA), enzyme immunoassay (ELISA) or else immunofluorescence.

These immunological detection methods, if indisputably they have good qualities, are not however entirely satisfactory.

In the first place, their sensitivity (which is defined by the minimum concentration of substance sought which these methods detect) is, in most cases, insufficient. Thus, BERCOFSKY and BERKOWER (*Antigen-Antibody Interaction*, In: WE Paul, *Fundamental Immunology*, RAVEN press, New York, 1984, 595) have shown that, as far as the detection of antibodies for example is concerned, with the exception of bacteriophage neutralisation tests with which it is possible to detect the presence of a single molecule of antibody but the use of which is extremely limited, very few methods have a sensitivity lower than 10 ng of antibody per ml of sample.

It is therefore desirable to develop new techniques which allow the detection threshold of a sought substance to be lowered.

Furthermore, all the immunological detection methods hitherto proposed include a stage which consists in incubating a pre-set volume - which is generally at the minimum of 500µl - of the sample for analysis with a specific reagent and to do this, for each substance sought. For this reason, they have the drawback of requiring, as soon as the analysis of a sample involves several substances - as is often the case in medical analyses for diagnostic purposes - a sample of relatively large volume, which is not always easily tolerated by patients, particularly in the case of blood samples.

Moreover, the fact that these detection methods require, for their implementation, the availability of the

sample for analysis or, at the very least, of a specimen of it, is not without imposing a certain number of constraints. Indeed:

- on the one hand, samples which have been subjected to analysis frequently have to be preserved so that the reliability of these analyses may subsequently be monitored or for additional analyses to be made. So, for example, blood transfusion centres, forensic medicine services and tissue sampling centres preserve specimens of all the biological samples that they are called on to take. This preservation, which is made by freezing said specimens, apart from being not insignificant in cost, requires adapted equipment and premises.

- on the other hand, samples can rarely be analysed at the place where they have been taken and it is often necessary to take them to the laboratory responsible for analysing them. In fact, transporting biological samples, apart from this never being very easy to implement given the short preservation period of biological substances in the absence of freezing, poses a certain number of difficulties when these samples are potentially contaminant. Moreover, the length of time taken to transport them differs by as much the obtaining of results from the analysis.

The problem arises, as a consequence, of supplying a method which makes it possible to detect the presence of a substance in a sample with, at the same time, very high sensitivity and high specificity, while offering the possibility of carrying out as many analyses as necessary from micro-samples, and to be free, moreover, from the constraints of preservation, despatch, and transportation of the samples presented by currently used methods for the detection of a substance, and which can, additionally, be

implemented easily and rapidly without requiring heavy and expensive equipment.

In fact, in the context of their work on the transmission of a biological activity in the form of an electromagnetic signal, the Inventors have noted that the effect of applying, to one and/or the other of the elements of a ligand-receptor pair such as an antigen-antibody pair, the electromagnetic signal characteristic of the biological activity of one and/or the other of these elements is, quite surprisingly, to amplify the formation of complexes between the two elements of this pair when these latter are set to react together, and this, very specifically, and have had the idea of capitalising on this effect in order to detect on the one hand, the presence of a substance in an analytical sample and, on the other hand, the presence of the electromagnetic signal characteristic of the biological activity of a substance in an electromagnetic radiation.

An object, therefore, of the present invention, is a process amplifying the formation between the two elements of a ligand-receptor pair by reaction of these two elements, which process is characterised in that it includes:

- bringing into contact the two elements of the ligand-receptor pair in conditions suitable to allow their reaction, and

- previously, simultaneously or subsequently to this bringing into contact, the application to one and/or the other of these elements of the electromagnetic signal characteristic of the biological activity of one and/or the other of said elements.

In terms of the present invention, by "ligand-receptor pair" is understood any pair formed by two substances

capable of recognising each other specifically, of binding and of reacting together forming complexes. Thus, it may be an antigen-antibody pair or a hapten-antibody pair in which the ligand (the antigen or the hapten) can be a biological compound (protein, enzyme, hormone, toxin, tumour marker, etc.), a chemical compound (medicinal active ingredient for example), or a cellular or particle antigen (cell, bacterium, virus, fungus, etc.), the receptor being able to be a soluble antibody or a membranous receptor. It may also be a pair formed by an enzyme or its specific substrate.

Furthermore, by "electromagnetic signal characteristic of the biological activity" of an element is understood the electromagnetic signal picked up from a biologically active element such as a substance, a cell or a micro-organism, etc., or from a material containing this element such as a purified preparation, a biological sample, an organ or a living being, as has been described in International Application WO 94/17406 in the name of J. BENVENISTE. By "electromagnetic signal characteristic of the biological activity" of an element is also understood the signals derived from a signal as defined above by signal digitisation and/or processing. Furthermore, in this expression, the term "characteristic" is used in the sense that the picked up electromagnetic signal contains information characterising the fact that the material from which this signal is picked up shows the biological activity in question. The electromagnetic signal picked up from a material containing a plurality of biologically active elements shows the biological activity of each of the elements that it contains.

According to a preferred first mode of implementation of the amplification process according to the invention, the reaction between the ligand and the receptor is

obtained by using two reagents containing respectively the ligand and the receptor, and to one and/or the other of these reagents is applied an electromagnetic test signal suspected to include the electromagnetic signal characteristic of the biological activity of this ligand and/or this receptor.

In what precedes and what follows, by the term "reagent" is denoted any preparation of which the composition is known, which contains the ligand or the receptor in an also known quantity and presents itself either in a dry form such as a lyophilisate to be reconstituted in a solvent, or in a liquid form such a solution or a suspension, the ligand and the receptor being able to be fixed on a solid phase (particles or beads of latex, glass or polystyrene etc.).

According to a first advantageous arrangement of this first mode of implementation, the application, to one and/or the other of the reagents, of the electromagnetic test signal is made by exposure of a solution or a suspension containing one and/or the other of these reagents, to this electromagnetic signal.

Alternatively, the application, to one and/or the other of the reagents, of the electromagnetic test signal is made by dilution of a solution or of a suspension including one and/or the other of these reagents, in a solvent having been previously exposed to this electromagnetic signal.

Thus, for example, when the reagents which it is required to use are in solution or in suspension in a liquid phase, it is possible to apply to them the electromagnetic test signal:

* either prior to their use:

- by exposing one and/or the other of these reagents or of the aliquots of one and/or the other of these reagents to this electromagnetic signal, or
- by diluting one and/or the other of said reagents or their aliquots in a volume of a solvent having been previously exposed to said electromagnetic signal,

* or during implementation of the amplification process according to the invention:

- by exposing to this electromagnetic signal an aliquot of each of these reagents, after placing these aliquots on a medium (plate for example) but prior to their being brought into contact, or
- by mixing an aliquot of the first reagent with an aliquot of the second reagent on a medium or in a tube, and by exposing this mixture to the electromagnetic signal, or else
- by mixing an aliquot of the first reagent with an aliquot of the second reagent on a medium or in a tube and by diluting this mixture in a volume of a solvent having been previously exposed to said electromagnetic signal.

According to another advantageous arrangement of this first mode of implementation, the application, to one and/or the other of the reagents, of the electromagnetic test signal is made by dissolution or putting into suspension this reagent or these reagents in a solvent having been previously exposed to this electromagnetic signal. This arrangement has a very particular advantage when the reagents which it is desired to use are in a dehydrated form such as a lyophilisate, since it is then possible to apply the electromagnetic test signal to them simply by dissolving them or by putting them in suspension depending on the case, in a volume of a solvent having been previously exposed to said electromagnetic signal.

To advantage, the electromagnetic test signal is an electromagnetic signal picked up from an analysis sample suspected to contain this ligand and/or this receptor, this sample being able to stem just as well from a biological sample (blood, urine, milk, etc.) as from a non biological sample (water, food product, pharmaceutical product, cosmetic product, etc.).

Alternatively, the electromagnetic test signal can also be an electromagnetic signal radiated by an electromagnetic radiation source, particularly a source suspected to emit radiation harmful to living beings such as the high voltage transmission line, transformer, electric motor, micro-wave oven, particle accelerator, X-ray source etc. Likewise, the electromagnetic test signal can stem from the acquisition of a mechanical signal like vibrations, an electrostatic signal or the like.

According to a second preferred mode of implementation of the amplification process according to the invention, the reaction between the ligand and the receptor is obtained by bringing into contact an analysis sample suspected to contain the ligand and/or the receptor, with a reagent containing either the receptor, or the ligand (according to the substance suspected to be present in the analytical sample with which it is desired to make this reagent react), and, to this sample and/or to this reagent, is applied the electromagnetic signal characteristic of the biological activity of said ligand and/or said receptor.

According to a first advantageous arrangement of this second mode of implementation, the application, to the analysis sample, of the electromagnetic signal characteristic of the biological activity of the ligand and/or the receptor is made by exposure of this sample to this electromagnetic signal or signals, or by dilution of

this sample in a solvent having been previously exposed to said electromagnetic signal or signals.

According to another advantageous arrangement of this second mode of implementation, the application, to the reagent intended to react with the analysis sample, of the electromagnetic signal characteristic of the biological activity of the ligand and/or the receptor is made by exposure of a solution or a suspension containing this reagent to this electromagnetic signal or signals, or by dilution of such a solution or suspension in a solvent having been previously exposed to this electromagnetic signal or signals, or again by dissolution or putting into suspension of this reagent in a solvent having been previously exposed to said electromagnetic signal or signals.

Alternatively, to the analysis sample and to the reagent intended to react with it, is applied the electromagnetic signal characteristic of the biological activity of the ligand and/or the receptor, by exposure of a solution or a suspension containing this sample and this reagent to this electromagnetic signal or signals, or by dilution of such a solution or suspension in a solvent having been previously exposed to said electromagnetic signal or signals.

According to a particularly preferred arrangement of this second mode of implementation, to the analysis sample and/or to the reagent intended to react with it, is applied both the electromagnetic signal characteristic of the biological activity of the ligand and the electromagnetic signal characteristic of the biological activity of the receptor. Indeed, the Inventors have noted that, if it is enough to apply, to the elements of the ligand-receptor pair, the electromagnetic signal characteristic of the

biological activity of a single one of these elements to obtain an amplification of the complexes formed by their reaction, this amplification is higher when the electromagnetic signals characteristic of the biological activity of each of them are applied to these elements simultaneously.

Whatever the mode of implementation of the amplification process according to the invention, the solvent having been previously exposed to the electromagnetic signal or signals is to advantage water or physiological solute.

Reagents able to be used in the amplification process according to the invention and containing the ligand on the one hand, and the receptor on the other hand, can just as well be ready-to-use commercially available reagents as reagents specially designed and prepared for the implementation of this process. Apart from the fact that, as mentioned above, these reagents can come in different forms (dry, liquid, etc.), they can, furthermore, be coupled to a marker such as a radioactive isotope, an enzyme, a fluorescent substance, a coloured particle, biotin or an organometallic compound, suitable to allow detection and/or measurement of the ligand-receptor complexes resulting form the reaction between the ligand and the receptor.

The amplification process includes to advantage, moreover, an acquisition stage of the electromagnetic signal characteristic of the biological activity of one and/or the other of the elements of the ligand-receptor pair.

As previously indicated, the electromagnetic signal characteristic of the biological activity of one and/or the other of the elements of the ligand-receptor pair can stem

either from an analysis sample suspected to contain this element or elements, or from an electromagnetic radiation source or from the acquisition of a mechanical (vibrations), electrostatic or other signal, or again from reagents containing the ligand or the receptor in solution or in suspension in a solvent, according to the modes of implementation of the amplification process according to the invention.

In a particularly advantageous way, the amplification process according to the invention also includes a recording and restitution stage of information representative of the electromagnetic signal characteristic of the biological activity of one and/or the other of the elements of the ligand-receptor pair. Thus, the electromagnetic signal characteristic of the biological activity of an analytical sample, once recorded, can be preserved indefinitely and used as often as necessary. Similarly, the electromagnetic signals characteristic of the biological activity of the ligand and of the biological activity of the receptor picked up from reagents, can be recorded once and for all and be used to obtain a plurality of reactions involving this ligand and this receptor.

The amplification process additionally includes to advantage a stage of detection of the complexes resulting from the reaction between the ligand and the receptor and, possibly, of measurement of these complexes. This stage can, to advantage, be completed by comparing the results obtained with those observed for a reaction serving as a "reference", that is to say a reaction conducted with the same ligand-receptor pair and in the same reaction conditions, but without application of an electromagnetic signal to the elements of this pair, whether previously,

simultaneously or subsequently to their being brought into contact.

The detection and/or measurement of the ligand-receptor complexes are able to be carried out by all the methods conventionally used to reveal and quantify the formation of such complexes. Thus, in the case of antigen-antibody complexes, it is possible just as well to use a revelation by agglutination, by immuno-precipitation, by fluorescence suppression, by fluorescence polarisation as a radio-immunological, immunoenzymatic test or else an immuno-fluorescence test.

According to a particularly preferred mode of implementation of the amplification process according to the invention, the ligand is an antigen or a hapten, whereas the receptor is an antibody or a membranous receptor targeted specifically against this ligand.

In a particularly advantageous way, the reaction between this ligand and this receptor is a reaction revealed by agglutination, given its simplicity and its speed of execution.

Another object of the present invention is a process for detecting the presence of a substance corresponding to one of the two elements of a ligand-receptor pair in an analytical sample, characterised in that it includes the implementation of an amplification process as defined above.

According to a particularly preferred first mode of implementation of this detection process, this includes:

- the bringing into contact of two reagents containing respectively the ligand and the receptor, in conditions suitable to allow their reaction,

- previously, simultaneously or subsequently to this bringing into contact, the application, to one and/or the

other of these reagents, of the electromagnetic signal characteristic of the biological activity of the analytical sample, and

- the detection and/or the measurement of the ligand-receptor complexes formed during the reaction between the two reagents.

Thus, obtaining an amplification of the formation of ligand-receptor complexes between the two reagents relative to a "reference" reaction (as previously defined) conveys the presence, in the electromagnetic signal of the biological activity of the analysis sample, of the electromagnetic signal characteristic of the biological activity of the substance sought and, as a consequence, conveys the presence, in this sample, of the substance sought.

In the event of such amplification being obtained and the analytical sample being able to contain not just one of the two elements of the ligand-receptor pair, but these two elements, the presence, in this sample, of the substance sought can be confirmed by comparing the results obtained with:

- either those observed for a reaction conducted in the same reaction conditions but with an application both of the electromagnetic signal characteristic of the biological activity of the analysis sample and of the electromagnetic signal characteristic of the biological activity of the ligand,

- or those observed for a reaction conducted in the same reaction conditions but with an application both of the electromagnetic signal characteristic of the biological activity of the analysis sample and of the electromagnetic signal characteristic of the biological activity of the receptor.

Thus, if the simultaneous application of the electromagnetic signal characteristic of the biological activity of the analytical sample and of the electromagnetic signal characteristic of the biological activity of the ligand is conveyed by an amplification of the formation of ligand-receptor complexes compared with the application of the single electromagnetic signal characteristic of the biological activity of said analytical sample, then this means that this sample does not contain a ligand and therefore contains only the receptor. It being the absence of an increase in the formation of ligand-receptor complexes that signals the presence of the ligand in the analytical sample.

Similarly, if the simultaneous application of the electromagnetic signal characteristic of the biological activity of the analytical sample and of the electromagnetic signal characteristic of the biological activity of the receptor is conveyed by an amplification of the formation of ligand-receptor complexes compared with the application of the single electromagnetic signal characteristic of the biological activity of said analytical sample, then it may be inferred from this that this sample does not contain a receptor and therefore contains only the ligand. It being the absence of an increase in the formation of ligand-receptor complexes that signals the presence of the receptor in the sample.

To avoid getting falsely negative results, that is to say results which would not make it possible to reveal an amplification effect of the application of the electromagnetic signal characteristic of the activity of the analytical sample and this, even though the latter contains in reality the substance sought, the concentrations of the ligand and of the receptor set to

react are chosen to advantage so as to be sufficient to lead to the obtaining of ligand-receptor complexes detectable in the absence of the application of the electromagnetic signal characteristic of the biological activity of said sample, but lower than the concentrations able to lead to a saturation of the reaction between this ligand and this receptor.

According to a second preferred mode of implementation of this detection process, this includes:

- the bringing into contact of the analytical sample with a reagent containing either the receptor, if the substance sought in the sample is the ligand, or the ligand, if the substance sought in the sample is the receptor, in conditions suitable to allow their reaction,
- previously, simultaneously or subsequently to this bringing into contact, the application, to this sample and/or this reagent, of the electromagnetic signal characteristic of the biological activity of the ligand and/or the receptor, and
- the detection and/or the measurement of the ligand-receptor complexes possibly formed, in which case, the obtaining of ligand-receptor complexes conveys the presence of the substance sought in the analytical sample.

This second preferred mode of implementation has a very particular advantage in detecting the presence of substances in samples, about which it is known that they are not detectable or only with great difficulty by the other available detection methods, since these substances are generally present in very low concentrations, indeed at trace level.

The process for detecting the presence of a substance in an analytical sample according to the invention has numerous advantages.

Indeed, on the one hand, it makes it possible to detect the presence of a sought substance with very great sensitivity and high specificity. Therefore, in the case, for example, of a bacteriological analysis, it makes it possible to eliminate the need to isolate the different germs, to cultivate them, to make an antibiogramme and to identify these germs by their biochemical, morphological and immunological character, and means that results can be obtained more rapidly than by the immunological detection methods currently used in bacteriology.

On the other hand, to the extent that it is enough to have a sample of the size of a drop to be in a position to acquire and record the electromagnetic signal characteristic of the biological activity of this sample and that, this signal, once recorded can be restored on request, this process offers the possibility of making as many analyses as desired from a microsample.

Lastly, the recording of an electromagnetic signal being able to be preserved indefinitely, for example in the form of an information file able to be preserved on a simple diskette or a CD-Rom, and to be transmitted from one place to another by any digital data transmission means, this process makes it possible, moreover, to eliminate all the constraints of preservation, despatch and transportation of samples presented by currently used methods for the detection of a substance.

This process is able to be used to detect any substance capable of binding specifically with another substance and of reacting with it, it being understood that the term "substance" as it is used here, denotes just as well a biological compound, a chemical compound, a cell as a micro-organism of the bacterium, virus or fungus type, knowing particularly that for any hapten, protein or

protein complex, it is possible to find on the market or to have manufactured the corresponding antibodies. By this token, this process finds, particularly, application in biological diagnostics, whether in human or veterinary medicine, or for the control of bacteriological quality in industries such as the pharmaceutical industry, the cosmetics industry, food production and industries.

A further object of the present invention is a process for detecting the presence, in an electromagnetic test signal, of an electromagnetic signal characteristic of the biological activity of a substance corresponding to one of the two elements of a ligand-receptor pair, which process is characterised in that it includes the implementation of an amplification process as defined above.

According to a preferred mode of implementation of this detection process, the electromagnetic test signal is the electromagnetic signal radiated by an electromagnetic radiation source.

Another object of the invention is a device for detecting the presence of a substance corresponding to one of the two elements of a ligand-receptor pair in an analytical sample, which device is characterised in that it implements a process according to the invention and in that it comprises:

- a) reception means of the analytical sample and of a reagent containing either the receptor, or the ligand, allowing them to be brought into contact in conditions suitable to allow their reaction;
- b) an electromagnetic signal source characteristic of the activity of the ligand and/or of the receptor;
- c) application means of the signal delivered by said electromagnetic signal source to the sample and/or the reagent; and

- d) detection and/or measurement means of the ligand-receptor complexes formed during the reaction between the sample and the reagent.

A further object of the invention is a device for detecting the presence of a substance corresponding to one of the two elements of a ligand-receptor pair in an analytical sample, which device is characterised in that it implements a process according to the invention and in that it comprises:

- a) reception means of two reagents containing respectively the ligand and the receptor, allowing them to be brought into contact in conditions suitable to allow their reaction;

- b) acquisition means of an electromagnetic signal of the analytical sample;

- c) application means of the signal delivered by said electromagnetic signal acquisition means to one and/or the other of the reagents; and

- d) detection and/or measurement means of the ligand-receptor complexes formed during the reaction between the two reagents.

According to an advantageous embodiment of these devices, the detection means comprise optical detection means.

In a preferred way, these devices comprise an enclosure fitted with an electrical and magnetic shielding surrounding said reception means.

Apart from the preceding arrangements, the invention includes still other arrangements which will emerge from the following supplementary description, which relates to embodiment examples of signal acquisition, recording and application devices able to be used according to the invention and to examples of experiments having allowed the

amplification process object of the present invention to be validated, and which refers to the appended drawings in which:

- Figure 1 shows a diagram of a first embodiment example of a signal acquisition device able to be used according to the present invention;

- Figure 2 shows a diagram of a second embodiment example of a signal acquisition device able to be used according to the present invention;

- Figure 3 shows a diagram of a first embodiment example of a signal recording device able to be used according to the present invention;

- Figure 4 shows a diagram of a second embodiment example of a signal recording device able to be used according to the present invention;

- Figure 5 shows a diagram of an embodiment example of a signal application device able to be used according to the present invention;

- Figure 6 shows a black and white image of 320 pixels x 240 pixels of the agglutinates formed during an agglutination reaction between the polysaccharidic antigen of *Escherichia coli* K1 and an antibody targeted against this antigen, after application of the electromagnetic signal characteristic of the biological activity of *Streptococcus*;

- Figure 7 shows a black and white image of 320 pixels x 240 pixels of the agglutinates formed during an agglutination reaction between the polysaccharidic antigen of *Escherichia coli* K1 and an antibody targeted against this antigen, after application of the electromagnetic signal characteristic of the biological activity of *Escherichia coli*;

- Figure 8 shows a black and white image of 320 pixels x 240 pixels of the agglutinates formed during an agglutination reaction between the polysaccharidic antigen of *Escherichia coli* K1 and an antibody targeted against this antigen, after simultaneous application of the electromagnetic signals characteristic of the biological activity of *Streptococcus* and of the biological activity of an antibody targeted against *Escherichia coli*;

- Figure 9 shows a black and white image of 320 pixels x 240 pixels of the agglutinates formed during an agglutination reaction between the polysaccharidic antigen of *Escherichia coli* K1 and an antibody targeted against this antigen, after simultaneous application of the electromagnetic signals characteristic of the biological activity of *Escherichia coli* and of the biological activity of its specific antibody; and

- Figure 10 shows a diagram of an embodiment example of a detection and/or measurement device of the ligand-receptor complexes able to be used according to the present invention.

In Figures 1 to 5 and 10, the same references have been used to denote the same elements.

Furthermore, each image in figures 6 to 9 corresponds to a surface of about 2 mm x 1·5 mm of the medium on which the agglutination reactions have been obtained.

It must be clearly understood, however, that these examples are given only as illustrations of the object of the invention and in no way constitute a restriction of it.

Reference is made first of all to Figures 1 to 5.

In Figure 1, a first embodiment example has been shown diagrammatically of an acquisition device of the electromagnetic signal characteristic of the biological activity of a substance 1 placed in a container 3, for

example a test tube. A sensor 5, typically a coil of the "telephone sensor" type marketed for the purpose of being applied to a telephone receiver and connected to a tape recorder, is applied against the container 3. The container 3 can also be constituted by a biological wall, particularly the skin of a living being. In such a case, the acquisition of the electromagnetic signal is made in a non-invasive way.

The signal picked up by the coil 5 is, to advantage, amplified by an amplifier 7 and is available at an output terminal 9. Without this presenting any kind of restrictive character of the example shown, a first end of the coil 5 is connected to the input of the amplifier-preamplifier 7, the opposite end being connected to a mass 11. In an embodiment example, the coil 5 is a commercially available telephone sensor having a length of 6 mm, an internal diameter of 6 mm containing a metal core, an external diameter of 16 mm and an impedance of 300 Ω.

In Figure 2 has been shown diagrammatically the preferred embodiment example of an acquisition device of the electromagnetic signal characteristic of the biological activity of a substance 1 contained in a container 3, in which the device includes, preferably, in an enclosure 13 fitted with an electrical and magnetic shielding, an irradiation transducer 15 of said substance 1 powered by a generator 17. The transducer 15 comprises, for example, a coil, to advantage completed by wave guides, for example an air gap (not shown) placed in contact with the external walls of the container 3.

The generator 17 generates a low frequency sinusoidal signal, low frequency square waves, pink noise or, to advantage, white noise. The excitation signal spectrum feeding the coil 15 corresponds approximately to the

spectrum of audible frequencies (20 Hz - 20 000 Hz). The generator 17 can be an analogue signal generator of known type or, for example, a read-only memory (ROM, PROM, EPROM, EEPROM in the terminology of the English-speaking world) containing the digital signal of the desired noise and which is connected to a digital-to-analogue converter, or the line output of a sound card of a multimedia microcomputer. However, the implementation of higher frequencies is not outside the framework of the present invention.

The acquisition sensor 5 can comprise a coil similar to the coil 5 of the device in Figure 1 or, to advantage, a small diameter coil connected by an electromagnetic wave guide to the wall of the container 3. To advantage, the signal picked up by the sensor 5 is available to an output terminal 9 of an amplifier-preamplifier 7.

The signal available at the terminal 9 may be directly applied to the substance or substances to be irradiated, particularly to the ligand, to the receptor or to the ligand-receptor pair (particularly by means of the device shown in Figure 5 and described below).

Recording the signal can be carried out in analogue by a signal recorder 19 (Figure 3), particularly on magnetic tape 21 adapted to the frequencies of the signal picked up. For acoustic frequencies, a tape recorder may particularly be used. The output terminal 9 of the signal acquisition device in Figures 1 or 2 is connected to the microphone input or to the line output of such a tape recorder. During playback, the signal is picked up at an output terminal 9', particularly at the line output or at the tape recorder loudspeaker output 19.

To advantage, a digital recording is made after analogue-to-digital conversion of the signal. A microcomputer 23 shown in Figure 4, fitted with a signal

acquisition card 25, is used for example. This can be for example a computer of the PC type, operating under the WINDOWS® 95 operating system of the MICROSOFT Company and comprising, apart from the acquisition card 25, a microprocessor 27, an input/output interface 29, a controller 31 of a file store 33 and a video interface 35 connected by one or more buses 37. The acquisition card 25 comprises an analogue converter 39 having, preferably, a resolution above 10 bits, for example equal to 12 bits, as well as a sampling frequency double the maximum frequency that it is wished to be able to digitise for the processing of signals. In the acoustic frequencies, the sampling frequency is to advantage approximately equal to 44 kHz. To process these types of signal, a micro-computer sound card is used to advantage, for example the Soundblaster 16 or the Soundblaster 32 card sold by the CREATIVE LABS Company. The computer 23 fitted with the restitution acquisition card 25, particularly of a Soundblaster 32 card can to advantage replace the signal generator 17 in Figure 2.

The output 9 of the signal acquisition devices in Figures 1 is connected to the input 9 of the analogue-to-digital converter 39 of the card 25 of the computer 23; the signal is then acquired for a period for example of between 1 and 60 s and the digital file is recorded in a file store 33 for example in the form of a WAV format sound file. This file may possibly be subject to digital processing, like for example a digital amplification for calibration of the signal level, a filtering for the elimination of undesired frequencies, or be converted into its spectrum by a discrete FOURIER transform, preferably by the fast FOURIER transform algorithm (FTT in the terminology of the English speaking world).

The sound reproduction time can be increased by repeating in a file several times a fragment or the totality of the original sound file.

On command, the possibly processed file is converted by a digital-to-analogue converter 41 of the card 25 (or of a separate card), which delivers at the output 9′ the electromagnetic analogue signal characteristic of the biological activity to be applied, according to the amplification process according to the invention, for example to an aliquot 43 of a first reagent and to an aliquot 45 of a second reagent, as shown in Figure 5.

To advantage, the application of the signal to these aliquots is made prior to their mixture. The medium on which these aliquots are placed, for example, a plate 47 fitted with a capillary 49 in the form of a coil, is placed in an electromagnetic field radiated by a transducer 51, typically a coil a first end of which 9,9′ is connected to the output 9 of an acquisition device of Figures 1 or 2 or to the output 9′ of a recording device of Figures 3 or 4. The coil end opposite the connection terminal 9,9′ is, for example, connected to the mass 11.

Without this having any kind of restrictice character, the transducer 51 comprises to advantage a coil, of horizontal axis, allowing the introduction of a plate 47. The coil has, for example, a length of 120 mm, an internal diameter of 25 mm, an external diameter of 28 mm, has 631 revolutions of a wire of a diameter of 0·5 mm and a resistance of 4·7 Ω.

To advantage, the electrical signal applied to this coil 51 will have an amplitude of 2 effective volts.

EXAMPLE 1: AMPLIFICATION OF THE FORMATION OF AGGLUTINATES BETWEEN THE POLYSACCHARIDIC ANTIGEN OF

***ESCHERICHIA COLI* K1 AND AN ANTIBODY TARGETED AGAINST THIS ANTIGEN**

The amplification process according to the invention has been validated by testing the effects, on an agglutination reaction between the polysaccharidic antigen of *Escherichia coli* K1 and an antibody targeted against this antigen:

- of the application of the electromagnetic signal characteristic of the biological activity of an antigenic substance alien to this reaction such as *Streptococcus*,

- of the application of the electromagnetic signal characteristic of the biological activity of *Escherichia coli*,

- of the simultaneous application of the electromagnetic signal characteristic of the biological activity of *Streptococcus* and of the electromagnetic signal characteristic of the biological activity of an antibody targeted against *Escherichia coli*, and lastly

- of the simultaneous application of the electromagnetic signal characteristic of the biological activity of *Escherichia coli* and of the electromagnetic signal characteristic of the biological activity of an antibody targeted against this antigen.

1) **Conducting tests**:

a) Acquisition of electromagnetic signals:

The acquisition of the electromagnetic signal characteristic of the biological activities of *Streptococcus*, *Escherichia coli* and of its specific antibody was made by means of the recording material in Figure 1.

The acquisition of the electromagnetic signal characteristic of the biological activity of *Streptococcus* was effected by placing at the centre of the enclosure 13 a

tube containing 1 ml of an aqueous suspension of previously formolated *Streptococcus* bacteria (6.10^7 bacteria/ml).

The acquisition of the electromagnetic signals characteristic of the biological activity of *Escherichia coli* and of its specific antibody was made by operating in the same way, but by using respectively:

- a tube containing 1 ml of an aqueous suspension of previously formolated *Streptococcus* bacteria (6.10^9 bacteria/ml).

- a tube containing 1 ml of a suspension of particles of a latex sensitised by a specific monoclonal mouse antibody of *Escherichia coli* K1, coming from a PASTOREX® MENINGITIS kit (Reference 61709 - SANOFI DIAGNOSTICS PASTEUR).

b) Preparation of the reagents of the agglutination reaction:

The tests were conducted by using as reagents:

- on the one hand, a solution of polysaccharidic antigen of *Escherichia coli* K1 prepared by dissolution of an antigen extract coming from a PASTOREX® MENINGITIS kit (Reference 61709 - SANOFI DIAGNOSTICS PASTEUR) in 1 ml of distilled and sterile water, then dilution to 1/7, 1/7·5, or 1/8 in a physiological serum; and

- on the other hand, the latex sensitised by a specific monoclonal mouse antibody of the antibody of *Escherichia coli* K1 present in this same kit, after dilution to 1/3 in a physiological serum.

c) Application of the electromagnetic signals to the agglutination reaction

For each of the tests, the following protocol was used:

- into an oven heated to 37°C is placed a transducer constituted by a coil measuring 120 mm in length and 25 mm

in internal diameter, having 631 revolutions and a resistance of 4.7 Ω, and connected to the output 9' of the digital-to-analogue converter 41 of a Soundblaster card of a computer 23 restoring the recording files constituted by the electromagnetic signal that it is desired to apply, the time needed to bring this transducer to the temperature of 37°C;

- on a plate fitted with a capillary in the form of a coil (of the type provided in PASTOREX MENINGITIS kits), at a short distance from the opening of the latter, is put a drop (i.e. 40 to 50 µl) of the antigen solution as described at point b) above, and a drop (corresponding also to a volume of 40 to 50 µl) of the latex sensitised by the antibody, taking care that these drops do not mix together;

- to the two drops of reagents so placed, is applied the electromagnetic signal or signals desired by placing the plate at the centre of the transducer for about 2 mn and by restoring a sound file by means of the computer 23 in figure 4,

- the two drops of reagents are mixed for about 10 seconds and for about 13 minutes in the oven the reaction mixture is left to migrate in the capillary and the agglutination reaction is left to happen;

- the plate is then taken out of the oven and this agglutination is then read.

As can be seen in Figure 10, this reading is taken by analysis, using analysis and image processing software run on a computer of the PC type 23' operating on the WINDOWS® 95 (MICROSOFT) operating system, of an image acquired using a video camera 53 positioned on an optical microscope 55 and connected to said computer by a video acquisition card 57. The camera 53 works in levels of grey. A first processing increasing the contrast, the threshold being

adjusted so that the agglutinates appear as black, whereas the zones devoid of particles of latex or of agglutinates appear as white.

From the analysis of the bidimensional spatial distribution of the dark zones of the image, the computer determines an agglutination index (I) calculated according to the formula:

$$I = \frac{\text{Surface taken up by agglutinates larger than 60 pixels}}{\text{Surface taken up by agglutinates equal to or smaller than 60 pixels}}$$

This agglutination index is higher in proportion to the size of the agglutinates formed during the agglutination reaction. The amplification is considered as positive when, during an experiment, the application of the electromagnetic signals characteristic of the biological activity of *Escherichia coli* and/or of the biological activity of its specific antibody leads to an agglutination index being obtained at least greater by 40% than the maximum agglutination index obtained, in the same conditions, and out of for example 3 experiments, after application of the electromagnetic signal characteristic of the biological activity of *Streptococcus*.

2) **Results**:

Table 1 below shows the agglutination indexes (I) obtained in a first series of tests aiming to compare the effects of the application of the electromagnetic signal characteristic of the biological activity of *Escherichia coli* to those observed after application, in the same reaction conditions, of the electromagnetic signal characteristic of the biological activity of *Streptococcus*, and this, for 3 different dilutions (1/7, 1/7·5, or 1/8) of

the solution of polysaccharidic antigen of *Escherichia coli* K1 used as a reagent in the agglutination reactions.

TABLE 1

Dilution of the E. coli K1 antigen solution	Agglutination index (I)	
	Streptococcus Signal	*E. coli* Signal
1/7	11 6 16	173 52 154
1/7·5	58 32 12	141 117 107
1/8	10 6 8	113 37 21

Furthermore, Figures 6 and 7 show, by way of examples, images of the agglutinates formed on the one hand, after application of the electromagnetic signal characteristic of the biological activity of *Streptococcus* (Figure 6) and, on the other hand, after application of the electromagnetic signal characteristic of the biological activity of *Escherichia coli* (Figure 7). These images correspond respectively to the agglutination indexes of 32 and 117 which are reported on the 5th line of results on Table 1.

Table 2 below shows, in its turn, the agglutination indexes (I) obtained in a second series of experiments in the context of which the effects of the simultaneous application of the electromagnetic signal characteristic of the biological activity of *Escherichia coli* and of the electromagnetic signal characteristic of the biological activity of the antibody targeted against *Escherichia coli*, were compared with those of the simultaneous application,

in the same reaction conditions, of the electromagnetic signal characteristic of the biological activity of *Streptococcus* and of the electromagnetic signal characteristic of the biological activity of the antibody targeted against *Escherichia coli*, and this, for 2 different dilutions (1/7, and 1/7·5) of the solution of polysaccharidic antigen of *Escherichia coli* K1 used as a reagent.

TABLE 2

Dilution of the E. coli K1 antigen solution	Agglutination index (I)	
	Streptococcus Signal + Anti-E.coli antibody Signal	*E. coli* Signal + Anti-E.coli antibody Signal
1/7	18 71	94 247
1/7·5	48 93	312 1141

Figures 8 and 9 show, also by way of example, images of the agglutinates which correspond respectively to the agglutination indexes of 71 and 247 reported on the 2nd line of results in Table 2.

All these results clearly prove the aptitude shown by the electromagnetic signal characteristic of the biological activity of one element of a ligand-receptor pair, to amplify the formation of complexes formed by the reaction between this ligand and this receptor and this, very specifically, since the electromagnetic signal characteristic of the biological activity of an element

which is biologically active but alien to this reaction does not produce an amplification effect.

They also show that this amplification is especially pronounced when, to the two elements of the ligand-receptor pair, is applied both the electromagnetic signal characteristic of the biological activity of this ligand and the electromagnetic signal characteristic of the biological activity of this receptor.

EXAMPLE 2: DETECTION OF THE PRESENCE OF ESCHERICHIA COLI IN A SAMPLE

The advantage of using the amplification process according to the invention to detect a substance present in an analytical sample was verified by conducting a series of tests with the aim of comparing the effects of the application, on an agglutination reaction between the polysaccharidic antigen of *Escherichia coli* K1 and a specific monoclonal mouse antibody of this antigen identical to that implemented in example 1 above, of the electromagnetic signal picked up from a sample of a food product, in the case in point stewed apples, previously contaminated by *Escherichia coli* bacteria, with those obtained during the application, in the same reaction conditions, of the electromagnetic signal picked up from a reference, or in other words uncontaminated sample of the same food product.

1) **Conducting tests**:

The acquisition of the electromagnetic signal of the samples of stewed apples (reference samples and contaminated samples) was made using the recording material in Figure 2, by placing at the centre of the enclosure 13:

- in the case of the reference samples, a tube containing 1 ml of stewed fruit previously diluted to 1/2 with physiological serum, and

- in the case of the contaminated samples, a tube containing 1 ml of stewed fruit previously diluted to 1/2 with physiological serum and contaminated, by addition of previously formulated *Escherichia coli* bacteria, at a rate of 3.10^{7} bacteria per ml of diluted stewed fruit.

The tests were conducted by using as reagents:

- on the one hand, a suspension containing *Escherichia coli* bacteria previously formulated in physiological serum, at a rate of 10^{7} bacteria/ml, and

- on the other hand, the latex sensitised by a specific mouse monoclonal antibody of the antibody of *Escherichia coli* K1 antibody present in this same kit, after dilution to 1/3 in physiological serum,

and by following an operating protocol identical to that described in paragraph c) of example 1 above.

2) **Results**:

Table 3 below shows the agglutination indexes (I) obtained in three series of tests.

TABLE 3

Tests	Agglutination index (I)	
	Reference samples	Contaminated samples
Series 1	10 25 27	42 93 104

Series 2	14 17 46	30 153 40
Series 3	19 34	34 314

As can be seen in Table 3, the size of the agglutinates formed during the reaction between the polysaccharidic antigen of *Escherichia coli* K1 and its specific antibody is substantially higher in the case where the electromagnetic signal applied during this reaction was picked up from a sample of stewed apples contaminated by *Escherichia coli* bacteria.

These results show that the amplification process according to the invention can to advantage be used to detect the presence, in an analytical sample, of a biologically active substance such as a bacterium, even when this sample has a complex composition, i.e. when it contains, as in the case of the samples of stewed apples, numerous other biologically active substances.

As emerges from what has been said previously, the Invention is in no way limited to the embodiments which have just been described in a more explicit way; it encompasses on the contrary all of its variants which can come to the mind of the technician in the field, without departing from the context, or the scope of the present Invention.